REMARKS

Claims 13, 38-43, 50, 53, 55, 60-71, 74 and 78-90 have been canceled.

Claims 1, 4, 6, 7, 10, 28, 31, 33, 34, 75-77, 91 and 92 have been amended. Claims 1, 6, 7, 10, 28, 33, 34, 59, 75, 76, 91 and 92 have been amended to specify a second animal species, rather than "one or more." Claim 10 has been further amended to conform to the language of claim 1 and to indicate that the cell line is "tolerated by said first and second animal species." Support for this latter language can be found at page 11, lines 14-20 of the specification. Claims 4, 7, 31 and 34 have been amended to indicate that the embryonic cells used in preparing the chimeric embryo are a mixture of embryonic cells. Claim 77 has been amended to clarify the language of the claim. Claims 59, 75-77, 91 and 92 have been amended to be consistent in language, i.e., to indicate that the embryonic cells of the first and second animal species cooperate in the formation of a further developing embryo, and to confirm to claims 1 and 28.

New claims 93-106 have been added. Support for claims 93-102 can be found in claims 4, 7, 31, 34, 59, 73, 75, 76, 91 and 92. Support for claims 103-106 can be found at page 4, line 19 – page 5, line 3 of the specification.

It is submitted that the above amendments do not constitute new matter and their entry is requested.

Claims 10 and 68 were rejected under 35 U.S.C. § 102(b) as being anticipated by ATCC entries HTB 157, 158 and 160. Claim 68 has been canceled. Claim 10 has been amended to indicate that the cell line is "tolerated by said first and second animal species." The first animal species is a human, and the second animal species is a non-human primate. As described at page 10, lines 14-20 of the specification, immune tolerance is measured by a mixed lymphocyte response. In order to anticipate claim 10, the cell line of the cited references must be tolerated by both a human and a non-human primate. Since the cited cell lines are human embryonic cells or human fetal cells, it is submitted that the cell lines would not be tolerated by a non-human primate as measured by a mixed lymphocyte response, and the Examiner has not provided any evidence that the cited cell lines would be so tolerated by a non-human primate. Accordingly,

ATCC entries HTB 157, 158 and 160 cannot anticipate claim 10. Withdrawal of this rejection is requested.

Claims 13, 66, 67 and 69-71 were rejected under 35 U.S.C. § 102(b) as being anticipated by Starzl et al. The cancellation of these claims obviates this rejection, and its withdrawal is requested.

Claims 83 and 86-90 were rejected under 35 U.S.C. § 102(b) as being anticipated by Starzl et al. The cancellation of these claims obviates this rejection, and its withdrawal is requested.

Claims 1, 28, 33, 34, 38-43, 59-65, 72-76, 79, 81, 82, 91 and 92 were rejected under 35 U.S.C. § 103(a) as being obvious over Gustafson. Claims 38-43, 60-65, 74, 79, 81 and 82 have been canceled. The claimed invention is directed to a chimeric embryo which comprises embryonic cells of a human and embryonic cells of a non-human primate. Such a chimeric embryo is neither disclosed nor suggested in Gustafson. Specifically, Gustafson teaches the preparation of hybrid embryos and their development. The hybrid embryos were prepared by mating white-faced ewes and an alpine doe to a fertile ram and buck, respectively. See, for example, page 267, description of "animals." Thus, the hybrid embryos of Gustafson were prepared by the sperm of one species fertilizing an egg of a second species. A hybrid embryo is not a chimeric embryo, which is made up of cells derived from two animal species, or as specifically claimed, is made up of embryonic cells from a human and a non-human primate. The fact that a hybrid embryo is not a chimeric embryo is further evidenced by Gustafson's use of the term "hybrid" **and not** the term "chimeric" in reference to the embryos produced and studied. Since Gustafson discloses the production of hybrid embryos, the most that Gustafson could suggest or could motivate a skilled artisan to produce would be to produce a hybrid embryo by (i) mating a human and a non-human primate, (ii) artificially inseminating a human or a non-human primate with sperm from a non-human primate or a human, respectively or (iii) in vitro fertilization of a human or a non-human primate egg by a non-human primate or a human sperm, respectively. Since the present invention is directed to a chimeric embryo, not a hybrid embryo, Gustafson does not render the claims obvious. Withdrawal of this rejection is requested.

The Examiner rejected claims 39-43, 55 and 85 under 35 U.S.C. § 112, first paragraph for lack of written description. The cancellation of these claims obviates this rejection, and its withdrawal is requested.

The Examiner rejected claim 78 under 35 U.S.C. § 112, first paragraph for lack of written description. The cancellation of this claim obviates this rejection, and its withdrawal is requested.

The Examiner rejected claims 10 and 13 under 35 U.S.C. § 112, first paragraph for lack of written description. Claim 13 has been canceled. Claim 10 has been amended to indicate that the cell line is "tolerated by said first and second animal species" as supported at page 11, lines 14-20. The first animal species is a human, and the second animal species is a non-human primate. As described at page 11, lines 14-20 of the specification, immune tolerance is measured by a mixed lymphocyte response. It is submitted that claim 10 as amended is fully described in the specification. Withdrawal of this rejection is requested.

The Examiner rejected claims 1, 3, 4, 6, 7, 10, 13, 28, 30, 31, 33, 34, 38-43, 50, 53, 55, 59-71 and 72-92 under 35 U.S.C. § 112, first paragraph for lack of written description. Claims 13, 38-43, 50, 53, 55, 60-71, 74 and 78-90 have been canceled. The presented claimed invention is directed to a human/non-human primate chimeric embryo (or cell line derived therefrom, for claim 10). Applicant submits that a skilled artisan can envision the detailed structure of the claimed chimeric embryo. The term "chimeric embryo" is used in the present specification in the same manner as used in the art. For example, Gilbert's *Developmental Biology* (Sinauer, 1997) describes a chimeric embryo to be: "the result of two or more early cleavage (usually 4- or 8-cell) embryos that have been artificially aggregated to form a composite embryo" (p. 187) and as being made from early stage embryo cells (blastomeres) and embryo stem (ES) cells (p. 189). In addition, Papaioannou and Gardner, (Papaioannou, V., and Gardner, R. L. (1979). Investigation of the lethal yellow Ay/Ay embryo using mouse chimaeras. *J Embryol Exp Morphol* **52,** 153-63) defines a chimeric embryo as being made from early cleavage embryos and inner cell mass cells. Thus, the chimeric embryo contains cells derived from two animal species (i.e., a human and a non-human primate, for the present application). Since the chimeric embryo contains cells from two animal species, a skilled artisan readily envisions that the chimeric embryo contains cells

from both species. Confirmation that a chimeric embryo contains cells from both species can readily be accomplished using conventional techniques, such as isolating DNA or mitochondria from individual cells of the chimeric embryo and analyzing for the parentage of the animal species used in preparing the chimeric embryo. Since a skilled artisan can readily envision the structure of a chimeric embryo, i.e., cells from two species, conception has occurred and reduction to practice has occurred by the filing of the present application. Accordingly, it is submitted that the specification provides an adequate written description of the invention claimed.

The Examiner rejected claims 1, 3, 4, 6, 7, 10, 13, 28, 30, 31, 33, 34, 38-43, 50, 53, 55, 59-71 and 72-92 under 35 U.S.C. § 112, first paragraph for lack of enablement. Claims 13, 38-43, 50, 53, 55, 60-71, 74 and 78-90 have been canceled. Applicant maintains that the technology for producing chimeric mammalian embryos is "robust". Applicant submits that the Examiner has not met her burden in demonstrating that the technology is not robust and is unpredictable, and that undue experimentation would be required.

Specifically, Applicant reiterates that the techniques for manipulating mammalian embryos are clearly robust and enabling for the production of chimeric embryos. For example, Hammer states in a 1998 retrospective (Hammer, R. E. (1998). *Egg culture: the foundation. Int J Dev Biol* **42**, 833-9):

> In 1963, Ralph [Brinster] reported a method for culturing eggs in micro-drops of medium under oil (Brinster, 1963), which has become universally used. Two years later, he identified pyruvate as the central and essential energy source for early stages of mouse eggs (Brinster, 1965b). These two developments revolutionized in vitro studies of mammalian eggs and issued in an era of intense research activity concerning egg culture and egg manipulation. Effective formulations of culture media could now be developed to allow routine in vitro maintenance of eggs, and important parameters for these recipes were soon determined ... **Thus, a foundation of understanding about the biology of early mammalian eggs was established between 1960 and 1970, and subsequent studies have broadened this understanding.** However, the greatest impact of a simple, reliable egg culture method has been to provide the ability to perform complicated manipulative procedures on preimplantation stages of

> mammalian embryos. In no area has this been more important than in development of transgenic animals. All methods for generating germ line genetic modifications rely on the ability to maintain and manipulate eggs and early developmental stages in vitro without loss of developmental competence. The importance of efficient egg culture to manipulation and transgenesis is fundamental and **enabling.** [Emphasis added].

Another recent article (Leoni, G. et al. (2000). Novel approach to cell sampling from preimplantation ovine embryos and its potential use in embryonic genome analysis. *J Reprod Fertil* **19**, 309-14) also speaks to the robustness of mammalian embryo manipulation techniques. It states:

> The major obstacle in the extensive analysis of the embryonic genome is the small number of cells typically obtained after the embryo biopsy. The object of the present study was to develop a simple approach that would allow the collection of a sufficient number of cells from a single embryo for use in further analyses... [N]o significant differences were found in the viability rates in vitro among blastocysts vitrified immediately after biopsy (77.8%), blastocysts .biopsied and vitrified after 24 h culture (76.9%) and blastocysts vitrified without manipulation (88.5%). In experiments in vivo, the lambing rate of biopsied and vitrified blastocysts was significantly ($P < 0.05$) lower (40.0%) compared with vitrified control embryos (68.7%). **This new approach to the biopsy of preimplantation embryos is a useful good model in the assisted reproductive technologies of domestic, wild and human species.** [Emphasis added].

An earlier report (Anderson, G.B. (1985). Manipulation of the mammalian embryo. *J Anim Sci* **61**, 1-13) shows that, by the early 1980s, the robustness of mammalian embryo manipulation techniques and their transferability across species lines was already part of the practice of the field:

> Technological advances in manipulation of mammalian embryos outside the maternal environment have resulted in opportunities for study of preimplantation embryo development, identification of developmental phenomena that are unique to mammals, and further improvement of technology. Mammalian embryos may be cultured in vitro at 37 C for up to several days or they may be stored at -196 C indefinitely. The mammalian embryo possesses the unique capacity to regulate its development and differentiate into a normal individual after being stimulated to incorporate foreign cells or after a portion of its cells are removed. **This regulatory**

> **ability has proven useful in research dealing with the production of chimeras... Some of these manipulations have been carried out primarily in laboratory mice, but as animal scientists identify beneficial uses in farm animals, these procedures are being extended to embryos of the large domestic species.** [Emphasis added].

The Examiner contends that only a very few species have been used in making chimeric embryos. Applicant respectfully submits that this is not the case: Picard, L. et al. (1990). Production of chimeric bovine embryos and calves by aggregation of inner cell masses with morulae. *Mol Reprod Dev* **27**, 295-304; Onishi, A. et al. (1994). Production of chimeric pigs and the analysis of chimerism using mitochondrial deoxyribonucleic acid as a cell marker. *Biol Reprod* **51**, 1069-75; Schoonjans, L. et al. (1996). Pluripotential rabbit embryonic stem (ES) cells are capable of forming overt coat color chimeras following injection into blastocysts. *Mol Reprod Dev* **45**, 439-43; Sumantri, C. et al. (1997). Fertility of sperm from a tetraparental chimeric bull. *Anim Reprod Sci* **46**, 35-45.

There is also evidence for intrauterine chimerism in the human, i.e., formation of a single individual from aggregation of blastomeres of fraternal twins: De la Chapelle, A. et al. (1974). Early fusion of two human embryos? *Ann Hum Genet* **38**, 63-75; Mayr, W.R. et al. (1979). Human chimera detectable only by investigation of her progeny. *Nature* **277**, 210-11.

The Examiner also states that none of the prior art enables one of ordinary skill in the art to culture primate embryos. Applicant respectfully submits that this is not the case: Pope, C.E. et al. (1982). Development of baboon preimplantation embryos to post-implantation stages *in vitro*. *Biol Reprod* **27**, 915-23; Gould, K. G. (1983). Ovum recovery and in vitro fertilization in the chimpanzee. *Fertil Steril* **40,** 378-83; Pope, V.Z. et al. (1984). SP-I secretion by baboon embryos *in vitro*. *Placenta* **5**, 403-12; Fourie, F.R. et al. (1987). Supplementation of Ham's F10 culture medium with three different sera in the culturing of baboon oocytes. *Comp Biochem Physiol A* **87,** 1103-6; and, Pope, C.E. et al. (1997). Birth of a western lowland gorilla (Gorilla gorilla gorilla) following *in vitro* fertilization and embryo transfer. *Am J Primatol* **41**, 247-60 all report the culture of primate embryos.

Machaty et al. (US 6,211,429) and Yanagimachi (US 6,376,743) claim methods of obtaining or producing mammalian embryos, respectively. The disclosures, and references cited therein, enable one of ordinary skill in the art to culture primate embryos, e.g., Homa, S.T., et al. (1994). *Hum Reprod* **9**, 2356-2361 and Herbert, M. (1995). *Hum Reprod* **10**, 2183-2186. Various methods are also disclosed for fusing and/or cooperatively aggregating two cell types together, e.g., Prather, R. (1996). *Proc Soc Exp Biol Med* **212**, 38-43 and Prather, et al. (1991). *Animal Applications of Research in Mammalian Development,* R.A. Pedersen, et al., Eds., The Cold Spring Harbor Laboratory Press, pp. 205-232. The various methods to culture primate embryos would have been well known to one of ordinary skill in the art. Chan, A.W.S. et al. (2000). Foreign DNA transmission by ICSI: injection of spermatozoa bound with exogenous DNA results in embryonic GFP expression and live Rhesus monkey births. *Mol Hum. Reprod* **6**, 26-33; Bavister, B.D.; Boatman, D.E.; Leibfried, L. et al. (1983). Fertilization and cleavage of rhesus monkey oocytes in vitro. *Biol Reprod* **28**, 983-999; Boatman, D.E. (1987). *In vitro* growth of non-human primate pre- and peri-implantation embryos. In Bavister, B.D. (ed.), *The Mammalian Preimplantation Embryo,* Plenum Press, pp. 273-308; Lanzendorf, S.E.; Zelinski-Wooten, M.B.; Stouffer, R.L., et al. (1990). Maturity at collection and the development potential of rhesus monkey oocytes. *Biol Reprod* **42**, 703-711.

Applicant's specification describes three specific technologies for making interspecific embryo chimeras, with citations to the published literature. The Examiner has cited dozens of references, establishing that the techniques are not only well known in the published literature, but readily apprehended and used by researchers in the art for a wide variety of investigations.

All early mammalian embryos, including human embryos, undergo the same initial developmental steps. All go through a *two cell, four cell,* and *eight cell* stage, and all are initially surrounded by an extracellular layer known the *zona pellucida.* All form a hollow *blastula,* containing an *inner cell mass.* The inner cell mass further develops into two or more layers of cells known as the germinal layers. The germinal layers, the ectoderm, the mesoderm, and the endoderm, give rise to the various cell types that make up the adult animal. (*An Introduction to Embryology,* Fourth Ed. (1975), Balinsky, B.I., W.B. Saunders Company, Philadelphia PA;

Molecular Biology of the Cell, Second Edition (1989), Alberts, B. et al., Garland Publishing, Inc., New York, NY). Applicant respectfully submits that methods to create chimeric embryos were adequately disclosed and enabled the present invention.

The Examiner maintains that intraspecies chimeras of mouse/rat and sheep/goat cannot be extrapolated to human/non-human primate chimeras. The Examiner states that, although human/chimp or human/gorilla may share greater than 90 percent DNA homology (actually 98% DNA homology); the DNA homology does not account for anatomical differences, differences in gestation, or more importantly, how to get a host mother to carry such a chimera. Applicant respectfully contends that the existing art was sufficient at the time of filing to permit one of ordinary skill in the art to construct the chimeric embyros, cell lines, and animals of the present invention.

Furthermore, the fact that the present specification is enabling not only for the production of human/non-human primate chimeric embryos, but also for the production of human/animal chimeric embryos, is illustrated by recent publications indicating that human/animal chimeric embryos are to be made. These publications are illustrated by DeWitt (*Nature* **420**:255, 2002) and Check (*Nature* **421**:4, 2003). Copies of these publications are attached. These publications indicate that techniques, such as those disclosed in the present application, will be used to create these chimeras. Thus, it is submitted that these publications provide further evidence that the present specification enables the claimed chimeric embryos.

In addition, the present claims are directed to a chimeric embryo comprising embryonic cells of a human and embryonic cells of a non-human primate which remain attached to one another and which cooperate in the formation of a further developing embryo. It is submitted that the scope of these claims is fully enabled by the specification. First, the specification (as demonstrated by the art) fully enables the making of claimed chimeric embryos. Second, the specification fully enables the use of the chimeric embryos, as demonstrated by the Examiner's conclusion that the chimeric embryos have utility for "(1) development toxicology assays; (2) studies of embryonic development disorders; (3) cryopreservation for future use." See page 30 of the Office Action.

Finally, Applicant submits that the specification is enabling for the use of human embryonic stem (ES) cells and maintains that undue experimentation would not have been required to produce human ES cells in view of the teachings and skill in the prior art. For example, non-human ES cells have been documented for the mouse (Martin, G.R. (1981). Isolation of a Pluripotent Cell Line from Early Mouse Embryos Cultured in Medium Conditioned by Teratocarcinoma Stem Cells. *Proc Natl Acad Sci USA* **78**, 7634-8), the pig (Wheeler (1994). *Reprod Fertil Dev* **6**, 563-8), the rhesus monkey (Thomson et al. (1995). Isolation of a primate embryonic stem cell line. *Proc Natl Acad Sci USA* **92**, 7844-7848), and the marmoset, another primate (Thomson, J.A. et al. (1996). Pluripotent Cell Lines Derived from Common Marmoset (*Callithrix jacchus*) Blastocysts. *Biol Reprod* **55**, 254-9), prior to filing the application. Armed with the background provided by this work, it is submitted that undue experimentation would not be required to practice the embodiments of the claimed invention pertaining to the production of chimeras from human embryonic cells and non-human ES cells. The mouse embryonic stem cells of these studies were established directly from normal preimplantation mouse embryos. The embryonic stem cells are pluripotent and were isolated from inner cell masses of late blastocysts. Eight embryonic stem cell lines from these studies were derived from common marmoset blastocysts. These embryonic stem cell lines were shown to differentiate into a number of different cell types. These teachings would have made the degree of experimentation required reasonable.

With regard to the generation of human ES cells, although not widely published at the time the application was filed, many groups were using the techniques employed for other mammals to develop human ES cell lines. During the year following filing of the present application, two groups published the isolation of such cell lines (Thomson, J.A. et al. (1998) Embryonic Stem Cell Lines Derived from Human Blastocysts. *Science* **282,** 1145-7; and Shamblott, M.J. et al. (1998). Derivation of Pluripotent Stem Cells from Cultured Human Primordial Germ Cells. *Proc Natl Acad Sci USA* **95,** 13726-31). These ES cell lines were isolated using existing techniques, without "undue experimentation." For example, in reporting the first primate ES cells, Thomson et al. stated: "The growth of monkey ES cells in culture

conditions that support feeder-dependent human EC [embryonal carcinoma] cells **suggests that similar conditions may support human ES cells,**" (Thomson et al. (1995) at p. 7848; emphasis added). That this understanding was correct was confirmed in the report by this group of the isolation of human ES cells, where it is stated: "five [human] ES cell lines originating from five separate embryos were derived, **essentially as described for nonhuman primate ES cells,**" (Thomson et al. (1998) at p. 1145; emphasis added).

Prior to the time of filing, at least one report appeared in the literature of an ES-like cell line derived from human embryos (Bongso, A. et al. (1994). Isolation and culture of inner cell mass cells from human blastocysts. *Hum Reprod* **9,** 2110-7). This report was referred to by Moreadith and Radford (1997. Gene targeting in embryonic stem cells: the new physiology and metabolism. *J Mol Med* **75**, 208-216) in the following fashion:

> The advent of techniques to generate gain-of-function and loss-of-function mutations in laboratory animals represents one of the major accomplishments in cell and molecular biology in mammals over the past two decades. Although the technology is generally limited only to the mouse at present, substantial effort is underway to develop these techniques, and to refine existing techniques, in other species. Putative pluripotential ES cell lines have been derived in a number of other species including hamster [70], pig [71–75], sheep [73], cattle [76], rabbit [77], rat [78], mink [79], monkey [80], and even humans [81]. Thus it seems likely the technology will be advanced into these additional species over the next few years, and each one of these may lend itself uniquely to problems ranging from development to tissue and organ physiology.

Reference [81] is Bongso et al. (1994). Moreover, it was generally known in the developmental biology community as of late 1997 that the Thomson group at the University of Wisconsin was working towards the isolation of human ES cells (their paper reporting this was published in 1998, and their 1995 paper reporting a primate stem cell line stated: "The growth of monkey ES cells in culture conditions that support feeder-dependent human EC [embryonal carcinoma] cells **suggests that similar conditions may support human ES cells**" (Thomson, 1995, p. 7848; emphasis added). Taken together, these reports and the discussion of Bosgso et al. (1994) by Moreadith and Radford (1997) indicates that by late 1997 knowledge of human ES

cells was available to researchers. Thus, Applicant submits that the existing art was sufficient at the time of filing to permit one of ordinary skill to obtain human ES cells without undue experimentation.

In view of the above remarks, it is submitted that the specification fully enables the chimeric embryos as set forth in the claims. Withdrawal of this rejection is requested.

The Examiner rejected claims 1, 3, 4, 6, 7, 10, 13, 28, 30, 31, 33, 34, 38-43, 50, 53, 55, 59-71 and 72-92 under 35 U.S.C. § 112, second paragraph for being indefinite. Claims 13, 38-43, 50, 53, 55, 60-71, 74 and 78-90 have been canceled. The term "chimeric embryo" is used in the present specification in the same manner as used in the art. That is, the chimeric embryo contains cells derived from two animal species (i.e., a human and a non-human primate, for the present application). Since the term "chimeric embryo" is used as in the art, it is submitted that it is definite to a skilled artisan. As emphasized in the claims, the cells of the chimeric embryo remain attached and cooperate in the formation of a further developing embryo. A skilled artisan readily recognizes that a developing embryo undergoes cell division and migration. Thus, a chimeric embryo is more than a mere mix of cells, as asserted by the Examiner.

Applicant has deleted the language "viable" (although Applicant submits that it was not used in a meaning repugnant to the normal meaning) and has canceled claims having the language "derived" or "originating." Applicant has also canceled claims having statements of use. Applicant has clarified the language of claim 10 to indicate that the chimeric embryo is comprised of embryonic cells and to set forth the embryonic cells. Claim 10 has also been amended to clarify that the cell line is tolerated by the first and second animal species. It is submitted that this term is definite. In addition, Applicant notes that the specification states that immune tolerance is measured by a mixed lymphocyte response. See page 11, lines 14-20 of the specification.

As previously discussed, the claims call for the embryonic cells of the first animal species and the embryonic cells of the second animal species to remain attached and to cooperate in the formation of a further developing embryo. By definition, the embryonic cells of both species must remain attached in order to have a chimeric embryo, i.e., an embryo comprising embryonic

cells of two animal species. Thus, the term "remaining attached" is definite to a skilled artisan. According to the definition of the term "embryo" provided by the Examiner, an "embryo" is "an organism in the early stages of development." Thus, "a further developing embryo" is implicit in the definition of "embryo." That is, there is no embryo if there are no stages of development. The specification clearly discloses that the embryonic cells of the first and second animal species "cooperate in the formation of a more developed embryo." See page 1, lines 19-20 of the specification. A more developed embryo is a "further developing" embryo. Also, as is clear from the meaning of "embryo," the embryo undergoes embryogenesis. According to *Stedman's Medical Dictionary, 26th Ed.*, page 559 (cited by the Examiner), "embryogenesis" is the "phase of development involved in establishment of the characteristic configuration of the embryonic body." Such "embryogenesis" or "further developing embryo" require cell division and migration as embryogenesis progresses. These features of an embryo are well known to a skilled artisan, and it is submitted that a skilled artisan fully understands the metes and bounds of the term "cooperate in the formation of a further developing embryo." Such features include cell division and some continued embryogenesis, as disclosed in the specification. Thus, it is submitted that this term is definite to one skilled in the art.

In view of the above remarks, it is submitted that the claimed invention is definite to a skilled artisan. Withdrawal of this rejection is requested.

The Examiner rejected claims 1, 3, 4, 6, 7, 10, 13, 28, 30, 31, 33, 34, 39-43, 59-67, 69-71, 72-83 and 86-92 under 35 U.S.C. § 101. Claims 13, 38-43, 60-67, 69-71, 74 and 78-90 have been canceled.

Applicant submits that it is the claims that define the patentable subject matter and not the specification. The claims are specifically directed to a chimeric embryo that is comprised of embryonic cells of a human and embryonic cells of a non-human primate. The claims require that the cells remain attached to one another and cooperate in the formation of a further developing embryo. Thus, the chimeric embryo contains human and non-human primate cells which cooperate in the formation of a further developing embryo. These features of the claimed invention are further pointed out at page 16, lines 13-19 and page 1, lines 19-20 of the

specification. The claims by their plain language do not encompass a human being. Furthermore, the claims by their plain language do not encompass a human embryo, as that term is conventionally used and understood, regardless of the issue of claim differentiation -- which is now moot. Finally, the claims are directed to subject matter clearly made by the hand of man. Applicant maintains that the subject matter claimed in the present invention is not a human being or a human embryo and that no statutory authority supports the rejection on these grounds.

The rejection is improper for two reasons: (1) it is not a proper statutory requirement for patentability; and (2) the claimed subject matter is not a human being or human embryo but rather, man-made chimeric embryos and cell lines derived from them. Applicant submits that the Commissioner has no authority to reject the claims of the present invention -- that are explicitly "made by man" -- on the grounds that they "embrace a human being."

As to the first point, the only issue is whether or not the claimed invention describes statutory subject matter. Nowhere does the statute restrict patentability based upon embracing a human being.

The Examiner recognizes that the Court in *Chakrabarty* (*Diamond v. Chakrabarty,* 447 U.S. 303 (1980)) held that statutory subject matter shall "include anything under the sun that is made by man." (at 309). The claimed subject matter is not naturally occurring. It is not disputed by the Examiner that the claimed subject matter is "made by man." Applicant claims a chimeric embryo or a cell line derived from the chimeric embryo. A human being is not claimed.

The Federal Circuit recently emphasized in *State Street Bank* (*State Street Bank & Trust Co. v. Signature Financial Group,* 149 F.3d 1368 (Fed. Cir. 1998)) that neither courts nor the Patent Office are authorized to embellish the statutory requirements for patentability. The Federal Circuit confronted the so-called "mathematical algorithm" and "business method" exceptions to patentability (at 1373, 1375-76):

> The repetitive use of the expansive term "any" in § 101 shows Congress's intent not to place any restrictions on the subject matter for which a patent may be obtained beyond those specifically recited in § 101. Indeed, the Supreme Court has acknowledged that Congress intended § 101 to extend to "anything under the sun that is made by man." *Diamond v. Chakrabarty,*

> 447 U.S. 303, 309, 100 S.Ct. 2204, 65 L.Ed.2d 144 (1980); *see also Diamond v. Diehr,* 450 U.S. 175, 182, 101 S.Ct. 1048, 67 L.Ed.2d 155 (1981).[3] Thus, it is improper to read limitations into § 101 on the subject matter that may be patented where the legislative history indicates that Congress clearly did not intend such limitations. *See Chakrabarty,* 447 U.S. at 308, 100 S.Ct. 2204 ("We have also cautioned that courts 'should not read into the patent laws limitations and conditions which the legislature has not expressed.' " (citations omitted)).
>
> [3] The Committee Reports accompanying the 1952 Act inform us that Congress intended statutory subject matter to "include anything under the sun that is made by man." S. Rep. No. 82-1979 at 5 (1952); H.R. Rep. No. 82-1923 at 6 (1952).

As the "embraces a human being" exception grafted by the Examiner in this case, these exceptions enjoyed no statutory sanction. Unlike the "embraces a human being" exception, they enjoyed prior judicial and Patent Office application in varying degrees.

As the Federal Circuit has held so clearly in *State Street,* "any" invention "made by man" is patentable subject matter. It is for Congress -- not the courts or the Patent Office -- to set forth any limitations on patentable subject matter. Congress has not established any limitation based on subject matter that "embraces a human being." The Commissioner lacks the authority to impose one under Section 101. Whether or not the Patent Office believes Congress intended to bar patentability of inventions that embrace a human being is not the issue. Congress has not done so expressly and the Patent Office has no authority to fill that gap.

Second, the Supreme Court has held that embryos, even those consisting exclusively of human cells, are not constitutionally protected as human beings *(see, Roe* v. *Wade,* 410 U.S. 113 (1973)). Congress -- in spite of almost 30 years of vigorous public debate -- has indicated no intention of altering this holding. That holding is mandatory authority and precludes the Examiner's finding that a single cell is sufficient to make a human being.

Embryos which are **not** exclusively human in origin, *viz.* the embryos of this invention, which contain human as well as non-human primate cells, are not human beings. They do not fall under 1077 OG 24 (4/21/87). Utility of such chimeric embryos as experimental models in biomedical and developmental biological research was documented in the original application.

Third, the present rejection is novel and unprecedented. As noted by the Examiner, mice and sheep have been engrafted with human bone marrow cells, and have been raised in laboratories as subjects of scientific investigations (Pixley, et al., (1994). *Pathobiology* **62**, 238-44; Almeida-Porada, et al., (1996). *Exp Hematol* **24**, 482-7).

Pixley et al. established long-term chimerism in normal mice transplanted *in utero* with human fetal hematopoietic stem cells. These human cells were injected into fetal mouse peritoneal cavities on days eleven through thirteen of gestation. These animals may develop and contain human cells in various organs. This engraftment of human cells into mouse fetuses does not now qualify the mouse as a human being, nor does it create a human being. The Office has never held that it does prior to the present invention and has regularly granted patents on such inventions.

Almeida-Porada et al. describes the transplantation *in utero* of pre-immune fetal sheep with human hematapoietic stem cells which result in a long term chimerism. These experiments reported the long term persistence of human cells in the human/sheep xenograft model. As with the above, the sheep, although containing human cells, are not considered human beings.

While Applicant disputes the Examiner's claim that Pixley et al. represents prior art with respect to the present invention, it is clear that these organisms represent "animals containing human cells." They are not constitutionally protected as human beings. They have no civil rights. They have no constitutionally recognized right of "reproductive choice." Because specific utility of chimeric embryos containing human cells, constructed by the methods of this invention, was documented in the original application, such organisms do not fall under 1077 OG 24 (4/21/87).

Applicant respectfully submits that a proportion of human cells in a chimeric embryo does not make that embryo a human being or a human embryo. In addition, the original application, and subsequent amendments, do not include any claims to a human being, but contains only claims to a chimeric embryo or a cell line derived from the chimeric embryo. The fact that a chimeric embryo or a cell line derived from the chimeric embryo has a human cellular component cannot exclude it from patentabllity, any more than the many patents that share that feature and have been awarded by the Patent Office. Subject matter consisting of, or derived

from, human cells in non-human animal systems has been, and continues to be, granted patents in the area of biotechnology.

Applicant maintains his position that he is not making a human being, or that his invention "embraces a human being." Applicant's invention is a chimeric embryo or a cell line that is developed in the laboratory and made by man. The "right to exclude others from making the invention" is the right to exclude others from making these chimeric embryos as described in the application. While each application is evaluated on its own facts, the Patent Office must provide a consistent interpretation as to what is patentable subject matter and what is not. Inventors routinely consult issued patents for guidance in determining the patentability of their own inventions.

The present claims are directed to a chimeric embryo. The present claims are not directed to a human being. As set forth in the definition of "embryo" in *Stedman's Medical Dictionary* (cited by the Examiner), an embryo is an organism in the early stages of development. It is implied in this definition that the embryo is not "the organism." Furthermore, the specification at page 16 does not state that the invention includes a human. It is submitted that the Examiner has read this portion of the specification entirely out of context in order to make the assertion she has made. The specification specifically states that the invention comprises, in part, "human embryos, human embryonic cells, and/or human embryonic stem cells **and** embryos, embryonic cells, and/or embryonic stem cells form on or more animal species, **which have been aggregated under conditions in which a viable enbryo forms**. (Page 16, lines 1-4, emphasis added) The clear reading of this portion of the specification is that a chimeric embryo is described, a description which is consistent with the remainder of the specification. The claims are clearly drawn to a chimeric embryo which by its definition cannot include an embryo of either donor species. Thus, the present claims are not directed to a human being, and it is submitted that the Patent Office has no authority to reject the present claims as limited to a chimeric embryo.

In addition, it is submitted that, even if the Patent Office is correct in its assertion that the ordinary canons of statutory construction support the interpretation of patentable subject matter

(a position which Applicant does not agree with the Examiner), the presently claimed subject matter is not directed to a human being and thus does not fall within such interpretation. Consequently, the Patent Office's position that claims to the chimeric embryos are not directed to patentable subject matter is not supported by any statutory basis or interpretation. As previously described, the present claims are directed to a chimeric embryo which comprises human embryonic cells and non-human primate embryonic cells. These cells aggregate and remain attached to cooperate in the formation of a further developing embryo. The chimeric embryo comprises cells of two species cooperating to form a developing embryo, and thus cannot be considered human. Such chimeric embryos are made by the hand of man and are encompassed within the terms "manufacture" or "composition of matter" as used in 35 U.S.C. § 101. These terms have been interpreted to include living things. *Diamond v. Chakrabarty*, 447 U.S. 303 (1980); *J.E.M. Ag Supply, Inc. v. Pioneer Hybrid Intl.*, 534 U.S. 124 (2001), *reh. den.* 122 S.Ct. 1600 (2002). Thus, it is submitted that the claimed chimeric embryos are directed to patentable subject matter, and since they are not a human being, there is no interpretation of the statute which would exclude them from being patentable subject matter.

In view of the above remarks, it is submitted that the rejection of the claims for lack of patentable subject matter is improper. Withdrawal of this rejection is requested.

The Examiner rejected claims 1, 3, 4, 6, 7, 10, 13, 28, 30, 31, 33, 34, 38, 39-43, 50, 53, 55 and 59-92 under 35 U.S.C. § 101 as not being supported by either a specific, substantial or credible asserted utility or a well established utility. Claims 13, 38-43, 53, 55, 60-71, 74 and 78-90 have been canceled.

As the Examiner is aware, only a single utility is required in order to meet the statutory requirement. This utility does not even need to be disclosed in an application, if it is known by a skilled artisan. The Examiner has enumerated ten utilities disclosed in the application at page 30 of the Office Action. Any one of these utilities is sufficient to meet the statutory requirement. Applicant submits that the first two enumerated utilities meet the statutory requirement. Since these two utilities meet the statutory requirement, Applicant will

not address the remaining enumerated utilities. However, Applicant disagrees with the Examiner's contention that many of these latter utilities are not credible.

The Examiner contents that the utility must be "specific." That is, the utility must be one "that would not generally apply" to all inventions of the same class, i.e., all embryos in the present case. There is simply no statutory basis or any basis in the case law for this requirement as interpreted by the Examiner. If there were a requirement for a specific utility of one invention not applicable to other inventions of the same field, then only truly pioneering inventions would meet this requirement. For example, a new compound having utility as an anti-hypertensive agent would not have a specific utility, since its utility would be applicable to all anti-hypertensive agents. Thus, it is submitted that a utility can be shared by other members of the same class of inventions and still be specific. In order to be a "general" utility, Applicant submits that the utility must be applicable to different classes of invention and not common to a single class of invention. For example, use as a "feedstock" would be a general utility, since a plant variety, a protein, nucleic acid, a hormone, a carbohydrate, a steroid, etc. could all be said to be useful as a "feedstock." In the present case, Applicant is not stating that a utility of the chimeric embryo is one that would be shared by many different classes of invention (i.e., a general utility). Instead, Applicant is stating that the utility is one that may be shared by a single class, e.g., embryos, it is nevertheless a specific utility. Consequently, Applicant submits that the utilities identified by the Examiner are specific.

In addition, it is submitted that the utilities are substantial, i.e., they have a "real world" use. Again, a utility of the chimeric embryo as a feedstock would not be a "real world" use. However, it is submitted that use of the chimeric embryos for toxicology assays and for studying embryonic developmental disorders are "real world" uses. Toxicology assays are a very critical step in the development of new drugs, and such assays represent a "real world" use. It has been established that certain new drugs may be found to be acceptable from an initial toxicity standpoint, but may turn out to have profound effects on a developing embryo and fetus. The chimeric embryos of the present invention can readily be used for toxicity assays. This use of these chimeric embryos will provide an assessment of drug toxicity not only on the developing

embryo but also on the cells of the animal species, i.e., human and non-human primate cells, of the chimeric embryo. The use of the chimeric embryos for toxicology assays would provide an additional test of a new drug's safety prior to its wide distribution. It is submitted that this utility is a "real world" use. Thus, it is submitted that the presently claimed invention has a substantial utility.

For the reasons discussed above, Applicant submits that the claimed chimeric embryos have at least one specific, substantial and credible utility. Withdrawal of this rejection is requested.

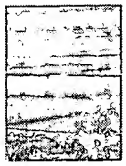
In view of the above amendments and remarks, it is believed that the present claims satisfy the provisions of the patent statutes and are patentable over the cited prior art. Reconsideration of the application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned to expedite the prosecution of the application.

<table>
<tr><td colspan="6">RESPECTFULLY SUBMITTED,</td></tr>
<tr><td>Name and Reg. Number</td><td colspan="5">Jeffrey L. Ihnen, Reg. No. 28,957</td></tr>
<tr><td>Signature</td><td colspan="2"></td><td>Date</td><td colspan="2">29 April 2003</td></tr>
<tr><td>Address</td><td colspan="5">ROTHWELL, FIGG, ERNST & MANBECK, pc
1425 K Street Street, N.W., Suite 800</td></tr>
<tr><td>City</td><td>Washington</td><td>State</td><td>D.C.</td><td>Zip Code</td><td>20005</td></tr>
<tr><td>Country</td><td>U.S.A.</td><td>Telephone</td><td>202-783-6040</td><td>Fax</td><td>202-783-6031</td></tr>
</table>

Attachments: DeWitt (*Nature* **420**:255, 2002)
Check (*Nature* **421**:4, 2003)

In the money
Congress backs financial boost for NSF
p257

Fair play
Revised misconduct guidelines issued for physicists
p258



Gene feast
Geneticists bowled over by sequences of rice chromosomes
p259

On guard
Conservationists target wildlife trade
p260

Biologists divided over proposal to create human–mouse embryos

Natalie DeWitt, New York

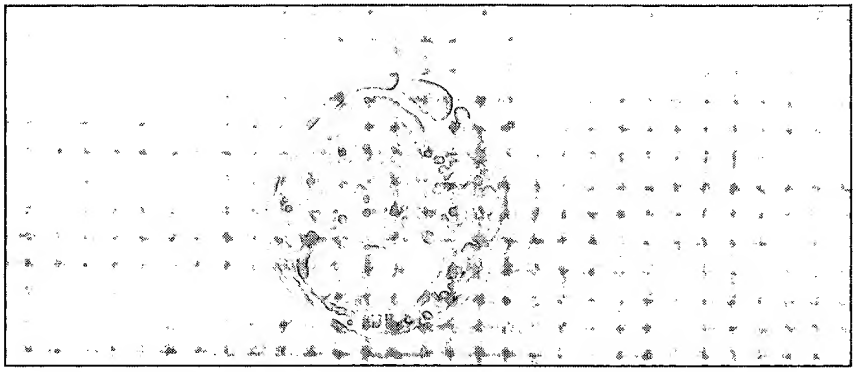
Human embryonic stem cells should be injected into mouse embryos to test the cells' likely clinical usefulness, some prominent US developmental biologists say. But the suggestion of creating such mixed-species embryos is likely to provoke public disquiet, and could galvanize political opposition to all research involving human embryos.

The idea was put forward on 13 November at a forum in New York, held to discuss standards for research with human embryonic stem cells. Ali Brivanlou, a developmental biologist at Rockefeller University in New York, and other supporters of the suggestion said that the 'chimaeric' embryos are needed to test the pluripotency of existing lines of human embryonic stem cells. Pluripotency — the ability to divide into cells of different functions, such as muscle or brain cells — is an important measure of the likely clinical usefulness of embryonic stem cells, a unique class of cells that can develop in different ways to perform many such functions.

But the forum, which was organized by the New York Academy of Sciences and Rockefeller University and included several US stem-cell researchers, failed to back a discussion document that would have included a call for the chimaeric embryos to be produced.

For years, mouse embryologists have tested pluripotency by injecting cells into a very early stage mouse embryo called a blastocyst, which consists of a hollow ball of cells. If the injected cells integrate into the embryo and contribute to the formation of every tissue, including the germ line which produces sperm and eggs, then they are pluripotent.

Ethical considerations would preclude an experiment that injected human cells into human embryos. But if they are compatible, cells from different mammalian embryos can be combined to form a viable chimaeric embryo. So some researchers are suggesting that the pluripotency of human embryonic stem cells could be tested by injecting them into mouse embryos. These embryos would then be reimplanted into a female mouse and allowed to develop. The tissues would be dissected and examined at various stages of development to see if they contained human cells.



D. SPEARS LTD/SPL

All mixed up: should human embryonic stem cells be fused with mouse blastocysts (pictured)?

But the New York meeting ended without agreement on the issue. Ronald McKay, a leading stem-cell researcher at the National Institute of Neurological Disorders and Stroke (NINDS) in Bethesda, Maryland, said that he flatly opposed a draft document circulated at the meeting that contained the chimaera proposal. "I am completely opposed to putting human embryonic stem cells into any condition that will cause moral affront," he said.

James Battey, a developmental biologist at NINDS and chair of the influential National Institutes of Health (NIH) Stem Cell Task Force, was excluded from a closed session held at the meeting on the discussion paper, and criticized participants for what he regards as excessive secrecy. Battey added that the group, which included prominent researchers such as Fred Gage of the Salk Institute for Biological Studies in La Jolla, California, and Harvard's Douglas Melton, was not fully representative of the field. James Thomson of the University of Wisconsin at Madison, who first isolated human embryonic stem cells, and Austin Smith of the University of Edinburgh, UK, were invited but did not attend, and human embryonic stem-cell experts from Australia and Israel were not invited.

During the open part of the discussion, Janet Rossant of the Mount Sinai Hospital in Toronto questioned whether making interspecies chimaeras was scientifically informative, claiming that in such experiments, "success is meaningful but failure is not". For one thing, she said, the different gestation periods for mice and humans may make it unlikely that the cells will combine in the embryo.

Rossant said there were viable alternatives to making interspecies chimaeras, such as assessing how the embryonic stem cells behave in culture, or testing whether they can engraft and form different tissues after injection into adult mice or mouse fetuses.

None of these tests would present the same ethical problems as producing chimaeras, Battey says. "Generally, the further along in development the fetus is, the less ethically complex these procedures are," he says.

Currently there are no NIH guidelines on any of these types of experiments. But an official at the agency says that they are generally viewed as ethically acceptable if they are done with embryos after the period of gonadal development has passed, so there was no danger, for example, of creating mouse sperm containing human DNA.

Experiments in which the cells of different species are combined in an embryo are likely to become more common as technology advances, researchers say. In September, Nissim Benvenisty of the Hebrew University of Jerusalem reported that he had grafted human embryonic stem cells into 1.5–2-day-old chick embryos (R. S. Goldstein, M. Drukker, B. E. Reubinoff and N. Benvenisty *Dev. Dynam.* **225**, 80–86; 2002). And Huizhen Sheng of Shanghai Second Medical University has transferred the genetic material of human cells into rabbit eggs (see *Nature* **419**, 334–336; 2002). □

Law sends laboratories into pathogen panic

Erika Check, Washington

When unknown parties mailed anthrax spores to several US addresses in the autumn of 2001, plant researchers at a herbarium at Harvard University began to get nervous.

For years, the researchers had stored a set of innocuous-looking brown envelopes that contained samples of anthrax. Within weeks of the attacks, President George W. Bush had signed a law called the USA Patriot Act, under which possession of anthrax without a "bona fide research justification" became a criminal offence. The Harvard researchers soon found themselves facing a tricky dilemma —how to balance their hoarding instincts against the new demands of homeland security.

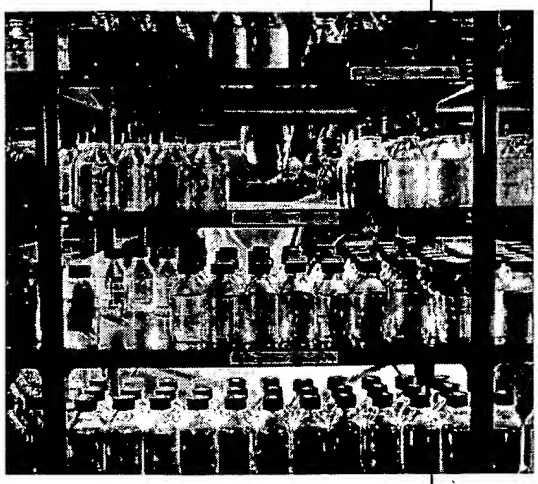
As thousands of US biologists face up to the same problem, some scientific leaders are concerned that researchers are dumping valuable samples to avoid trouble with the law.

For example, Ron Atlas, president of the American Society for Microbiology, is alarmed by the prosecution last July of a University of Connecticut graduate student who kept anthrax in his freezer. Atlas says these old microbes could hold useful information. "We are really in a delicate balance as to whether individuals will hold on to their cultures or whether they'll feel endangered by the USA Patriot Act," he says. He also warns that those who clear out their freezers may have problems restocking them because of new restrictions on the movement of pathogens between labs.

Some institutions — such as Iowa State University at Ames, which destroyed its entire archive of anthrax samples in October 2001 — have ordered mass clear-outs of materials. But individual researchers have also taken it upon themselves to dump potentially dangerous microbes. John Collier, a Harvard microbiologist who has long worked on the anthrax toxin, got rid of his samples of the bacteria late in 2001. "I wanted to be able to tell the world we didn't have *Bacillus anthracis*," Collier says.

The issue has now attracted the White House's attention — in part because archived samples could prove useful in criminal investigations. Kathryn Harrington, a spokeswoman for the administration's Office of Science and Technology Policy, says that the office is "aware of the destruction of select agents and is concerned". She adds that the office is trying to "encourage researchers to transfer materials to a secure facility, rather than destroy them".

This is exactly what the Harvard plant scientists did, after consulting their colleague molecular biologist Matthew Meselson. He contacted Paul Keim, a microbiologist at Northern Arizona University in Flagstaff who has spent his career studying anthrax. Keim persuaded the Harvard scientists to send him their envelopes, which are thought to contain anthrax taken from the blood of a cow in 1883. "We were interested in these for basic pathogen-evolution studies," Keim says. "But now they're crucial for fighting bioterrorism."



Bottling it: biologists are jettisoning their collections to avoid falling foul of legislation.

G. TOMPKINSON/SPL

Keim also argues that the federal government should use some of its new bioterrorism funds to solve the problem once and for all by creating a central repository for pathogens. "There's a big problem with saving these collections and a big problem with getting access to them," Keim says. "What's the point of putting $1.7 billion into the research if nobody can get hold of the strains?" ■

Biotech critic tries to sew up research on chimaeras

Erika Check, Washington

Scientists who are seeking to meld human embryonic stem cells with mouse embryos have been warned that they could be sued if they pursue the idea.

The 'chimaeric' embryos would be used to test the stem cells' ability to divide into cells with different functions (see *Nature* 420, 255; 2002). But Jeremy Rifkin, an economist and well-known critic of the biotechnology industry, has told researchers to abandon their plans, claiming that he is about to win a wide-ranging patent on human–animal chimaeras.

"They're saying they cannot take advantage of therapeutic cloning with stem cells unless they place them in an animal model," Rifkin says. "And we're saying we control that."

Rifkin and Stuart Newman, a cell biologist at New York Medical College, applied for the patent in 1997. So far, examiners at the US Patent and Trademark Office have said three times that the application should be turned down — but it remains under review. Rifkin claims that he will prevail in a court appeal even if the patent office denies his claims.

Rifkin's lawyers have sent letters asserting the claims to prominent researchers in the field, including Ali Brivanlou, a developmental biologist at Rockefeller University in New York, Austin Smith of the University of Edinburgh, UK, and James Thomson of the University of Wisconsin at Madison.

But Brivanlou, one of the researchers working on a discussion paper considering the production of chimaeric embryos, says he is undeterred by the letter. "This certainly isn't going to stop me from doing anything," he says. "I'm not taking it seriously."

Brivanlou says that he is highly sceptical of Rifkin's warnings. He points out that Rifkin has not been awarded a patent and that Rifkin and Newman have not done the experiments described in their patent application as proof that it is possible to make a chimaeric embryo.

Rifkin and his lawyer contend that they don't have to make a chimaera to win a patent on it. "There is no rule, regulation, case law or statute of which I'm aware that requires the inventor to practise his or her invention," says Patrick Coyne, Rifkin's lawyer at the Washington firm Collier Shannon Scott.

A lawyer not associated with the case says that although this is technically correct, courts have recently asked for proof that biotechnology inventions actually work before granting patents on them. ■